Use of Azolla (Azolla pinnata) Meal as a substitute for defatted Soybean Meal in diets of Juvenile Black Tiger Shrimp (Penaeus monodon).

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Original paper

USE OF AZOLLA (*Azolla pinnata*) MEAL AS A SUBSTITUTE FOR DEFATTED SOYBEAN MEAL IN DIETS OF JUVENILE BLACK TIGER SHRIMP (*Penaeus monodon)*

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ABSTRACT

A 42-day feeding experiment was conducted to study the feasibility of utilizing azolla (Azolla pinnata) meal (AZM) as a replacement for soybean meal (SBM) in the diets for juvenile Penaeus monodon. The replacement levels of azolla meal protein in the diets were 0, 25, 50, 75, and 100% of the total soybean meal protein. All diets were isonitrogenous at 40% crude protein. Shrimp (mean initial weight, 0.49±0.02 g) were fed three times daily ad libitum at an initial feeding allowance of 10% total body weight per day. A completely randomized design was used in the study and shrimp were stocked at a density of 10 animals/72 L-tank in triplicates. There were not significant differences in weight gains (1.97-2.06 g), specific growth rates (SGR 3.81-3.89%/d), feed conversion ratios (FCR 2.06-2.77), protein efficiency ratios (PER 0.89-1.24), apparent protein utilization (APU, 43.3–56.7%), and survival rates (99.1-100%) among shrimp groups (P>0.05). Feeding preference tests showed that either soybean meal-based diet or azolla meal-based diet was similarly preferred (P>0.05) by shrimp with the preference values of 51 and 40% for soybean meal-based diet and azolla meal-based diet, respectively. The results of this study suggest that azolla meal protein can replace up to 100% of the soybean meal protein in practical diet for juvenile black tiger shrimp Penaeus monodon under laboratory conditions without any adverse performance. The use of azolla meal as an alternative plant protein source to soybean meal may be able to reduce the feed costs for Penaeus monodon aquaculture.

Key words: Azolla pinnata meal; soybean meal; Penaeus monodon; practical diets; juvenile

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INTRODUCTION

When global fishmeal production decreases and fishmeal prices become more expensive, nutritionists seek less expensive plant protein sources such as soybean meal (Akiyama, 1991; Garcia-Ulloa *et al*., 2003; Du and Niu, 2003), lupin meal (Sudaryono *et al*., 1999a, 1999b, 1999c), canola (rapeseed meal) (Thiessen and Maenz, 2005), linseed meal (El-Saidy and Gaber, 2001), and legumes (De Silva *et al*., 1988; Hossain *et al*., 2001) to replace fishmeal in formulated diets. Those plant meals have been reported to be suitable as partial replacement for fishmeal in aquaculture feed formulations.

Of plant protein sources, soybean meal (SBM) is the most widely utilized plant protein source due to its nutritional quality, favorable cost, abundant and sustainable availability (Akiyama, 1991). The success of using soybean meal as a major plant protein

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source in diets for freshwater prawn, *Macrobrachium rosenbergii* has been reported by Koshio *et al.* (1992), Tidwell *et al.* (1993), Zhu and Yang (1995), and Dong and Niu (2000). However, high substitution levels of soybean meal proteins in fish and marine shrimp diets seem to affect palatability, leading to reduced food consumption, poorer growth and lower feed efficiency (Lim and Dominy, 1990; Tacon and Akiyama, 1997; Floreto *et al*., 2000). Soybean is inadequately grown in the tropical countries and in recent years the cost of imported SBM has continued to rise due to increase in global demand from the animal feed industry and direct use in human foods (Ng and Chen, 2002). To reduce the possible negative effects of such changes, the replacement of soybean protein with more economical, alternative sources of protein is desirable, as long as satisfactory growth and feed efficiency can be maintained.

Azolla pinnata is, from Pteridophyta Division, one of aquatic plants living all over the year in lake, paddy field, freshwater pond areas, river or irrigation channel (Lumpkin and Plucknett, 1982). Khan (1988) reported that *Azolla* sp. can produce 450-600 kg N_2/ha/year. The production of *A. pinnata* is high around 1000-2000 kg/ha/day and it is similar to the production of 10-30 kg protein (Lumpkin and Plucknett, 1982).

The aim of this study was to evaluate the feasibility of replacing defatted soybean meal (SBM) with azolla meal (AZM) in practical diets on growth performance and nutrient utilization of juvenile *P. monodon* cultured in laboratory.

MATERIALS AND METHODS

Experimental diets

All feed ingredients used in this study other than azolla meal were obtained from commercial suppliers in Semarang, Central Java Province. Azolla meal was prepared in the laboratory obtained from aquatic plant of *Azolla pinnata* living in water irrigation of paddy field in Semarang. The raw materials of *A. pinnata* were dried overnight at 55°C using an oven.

The resultant dry components were then finely grounded using an electric grinder and passed through a 0.5 mm mesh sieve.

In preparing the diets, all dry feed ingredients were first grounded to a small particle size in a laboratory electric grinder and sieved through an approximately 250 μm sieve. Ingredients were thoroughly mixed in a commercial food mixer for 15 minutes, after oil was gradually added while mixing constantly. Enough water was slowly added to make a stiff dough. The wet mixture was steamed for 5 min and the diets were produced in a noodle-like shape of 2.0 mm in diameter using a meat grinder. Then the pelleted diets were dried overnight at 55°C. After drying, the diets were broken up, sieved into appropriate pellet sizes, packed in plastic bags and stored in a freezer until used in feeding trials.

Five isonitrogenous experimental diets containing approximately 40% crude protein (dry weight basis) were formulated by replacing 0, 25, 50, 75, and 100% of the protein from soybean meal with protein from azolla meal (**Table 1**). Crude protein at 40% in the diet is reported to be the optimal level for juvenile *P. monodon* (Alava and Lim, 1983; Shiau *et al.*, 1991). The diet containing no azolla meal was considered to be the control diet (D0) for this study. The three animal protein sources (fish meal, squid meal, krill meal) in all diets were included at the same levels. α-starch and filler (α-cellulose) contents were adjusted to maintain similar dietary protein contents. A 4% vitamin/mineral mix, 4% pollack liver oil, 1% cholesterol, 1% soybean lecithin, and 2.5% carboxymethylcellulose (CMC) as a binder were added to each diet. The amount of feed ingredients included in all formulated diets was within the ranges commonly used in commercial feed (Akiyama, 1991; Akiyama and Dominy, 1991).

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The samples from each batch were pooled and analyzed for proximate composition upon termination of the study (**Table 1**). All diets were analyzed for dry matter by using an oven, ash by using a muffle furnace, crude protein by using the Kjeldahl method, and crude lipid by extracting with anhydrous ether in a Soxtec following the standard methods of Association of Official Analytical Chemists (AOAC, 1990).

Culture condition

Juvenile black tiger shrimp *Penaeus monodon* were obtained from a local shrimp hatchery and acclimated to laboratory conditions for 2 weeks in two-500-L circular fiberglass tanks. During this period, shrimp received a commercial feed. At the beginning of the experiment, 15 72-L rectangular blue plastic tanks were each stocked with 10 shrimp with an initial average weight 0.49±0.02 g. The tanks were supplied with aerated and filtered seawater at a rate of 250 mL/min using a flow-through water system. The tanks were substrate-free flat-bottom plastic tanks equipped with continuous aeration and a black plastic mesh lid to minimize disturbances and prevent shrimp from jumping out. Five experimental diets were assigned to triplicate tanks in a completely randomized design.

Table 1. Composition of experimental diets (% as fed basis)

Ingredient	Replacement of dietary protein of soybean meal by azolla meal				
	0% (D0)	25% (D25)	50% (D50)	75% (D75)	100% (D100)
Defatted soybean meal	300	225	150	75	0
Azolla meal	0	120	240	360	480
Fish meal	230	230	230	230	230
Squid meal	50	50	50	50	50
Krill meal	50	50	50	50	50
Pollack liver oil	40	30	20	10	0
Cholesterol	10	10	10	10	10
Soybean lecithin	10	10	10	10	10
α-Starch	180	160	140	120	100
Vitamin mix	20	20	20	20	20
Mineral mix	20	20	20	20	20
Cr_2O_3	5	5	5	5	5
Carboxymethylcellulose	25	25	25	25	25
Filler (a-cellulose)	60	45	30	15	0
Proximate analysis (% dry matter basis)					
Moisture	7.81	5.54	6.28	6.32	6.80
Ash	9.78	12.36	14.23	17.60	20.07
Crude lipid	9.75	9.40	9.28	9.57	9.72
Crude protein	41.72	40.44	42.16	41.12	41.34

Prior to start of the feeding trial, shrimp were acclimated to experimental diets and conditions for a 7-d period. Shrimp were fed three times daily *ad libitum* at 08.00, 13.00, and 17.00 on an initial feeding allowance of 10% total body weight/d for 42 d. Any mortalities during the experiment were replaced by tagged shrimp from the reserve tank to avoid density dependent effects, but no measurements were made for replacement shrimp. All shrimp in reserve tanks were fed with a commercial diet prior to being introduced into experimental tanks. The amount of feed given to each tank was determined for each group of ten shrimp. It was assumed that all shrimp in one tank

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consumed an equal amount of offered feed, but the proportion of the feed consumed by the replacement shrimp was not taken into account for determination of estimated feed intake.

The wet weights of individual shrimp were recorded at every 14-d interval to adjust the amount of feed given. Shrimp were allowed to feed for 4 h and then the uneaten food remaining on the bottom of the tanks was siphoned out, separated from other waste materials (faeces and exuvia), collected, redried, and weighed for determination of the total estimated feed intake. The percentage dry matter loss of each diet immersed in seawater for up to 4 h was used as a correction factor for calculation of the total estimated feed intake in this present study. All tanks were cleaned before every feeding.

The feeding trial was conducted at ambient temperature and subjected to natural photoperiod (approximately 12-h light/12-h dark during the experiment). Water quality parameters were assessed every 3 d. Water temperature ranged between 26.1 and 29.0°C, salinity 32.5-35 ppt, dissolved oxygen $\geq$ 5.0 mg/L, and total ammonia nitrogen < 0.01 mg/L.

Feeding preference test

The feeding preference test for juvenile *P. monodon* as previously conducted by Sudaryono (1998) and a modification of the method as described by Hartati and Briggs (1993) was employed in the present study to compare the attractiveness of soybean meal based diet (no azolla meal) and azolla meal based diet (no soybean meal). Eight replicate groups of ten juvenile *P. monodon*, mean weight 0.97±0.13 g, were tested in separate glass tanks (35x20x25 cm^3) equipped with aeration. Each tank was divided by a vertically sliding plexiglass wall into two equal compartments, "preparation" compartment and "testing" compartment. The "testing" compartment was divided into two freely available, equal section (X and Y) by a piece of black acrylic plate measuring 12 cm^2. All walls of the tank were covered with black plastic sheet to avoid any disturbances. However, one wall of the testing section was left uncovered to enable observation of the response of shrimp to tested diets. Approximately 160 shrimp were acclimated for a week in a common holding tank equipped with an aeration before being allocated into the experimental tanks. Shrimp were fed a commercial diet during the acclimatization period and starved for 48 hours prior to the trial.

At the beginning of the test, soybean meal based diet at the amount of 20% wet body weight was introduced into section X, while the same amount of azolla meal based diet was introduced into section Y. At the start of the trial, the sliding wall in the middle of the tank was opened to allow shrimp attend to each test diet. Aeration was turned off during the trial. Number of shrimp located in each compartment was counted every minute for a period of 30 minutes. The number of shrimp feeding on each diet, each minute for 30 minutes was used as a unit to measure the degree of attractiveness of the diets (Hartati and Briggs, 1993). The analyzed data were then converted into data giving the percentage of shrimp responding to the diets out of the total number of shrimp responding during the test.

Performance evaluation

Biological parameters used to evaluate the quality of experimental diets were calculated by equations as follows (Shiau *et al.*, 1991):Weight gain (g) = (final – initial) wet weight; Specific growth rate (SGR, %/day) = 100 x [(ln final weight – ln initial weight) / time (days)];

Estimated total dry feed intake (g/shrimp) = dry feed given – dry remaining feed recovered;

Feed conversion ratio (FCR) = estimated dry feed intake/wet weight gain;

Protein efficiency ratio (PER) = wet weight gain/dry protein intake;

Apparent protein utilization (APU, %) = (dry protein gain/dry protein intake) x 100

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Statistical analysis
All data were statistically analyzed by the Statistical Analysis Program of SPSS Inc. One-way ANOVA and the Duncan's multiple comparison test at P<0.05 were used to compare the observed values between diets. Covariate ANOVA was used to demonstrate that there was no effect of initial shrimp weight on the observed parameters. All percentages and ratio data were transformed to arcsine values before analyzed (Zar, 1984).

RESULTS AND DISCUSSION

Results

Table 2 shows the growth performance, feed utilization efficiency, and survival of the juvenile black tiger shrimp *P. monodon* fed the diets containing different replacement levels of protein of soybean meal with azolla meal for 42 d. Survival was very high and similar in every dietary treatment, more than 99% (P>0.05). There were no significant differences (P>0.05) in the initial weights of shrimp stocked (0.49-0.50 g), weight gain (1.89-2.06 g), specific growth rate (SGR, 3.77-3.89%/d), feed intake (4.06-5.54 g/shrimp), feed conversion ratio (FCR, 2.06-2.77), protein efficiency ratio (PER, 0.89-1.24), and apparent protein utilization (APU, 43.3-56.7%) among groups of shrimp.

The feeding preference test for the two diets containing 100% soybean meal (D0) and 100% azolla meal (D100) showed that there were no differences in attractiveness between the soybean meal based diet with a preference value of 51% and azolla meal based diet with a preference value of 40% (**Table 3**).

Table 2. Growth response and feed utilization efficiency of shrimp fed diets containing various replacement levels of protein of soybean meal by azolla meal for the 42-day feeding period. Values reported are means ± SD of three replicates. Values in the same row with the same superscript letters are not significantly different (P>0.05).

	% Replacement of dietary protein of soybean meal by azolla meal				
Parameters [1]	0 (D0)	25 (D25)	50 (D50)	75 (D75)	100 (D100)
Initial body weight (g)	0.50 ± 0.02^a	0.49 ± 0.02^a	0.50 ± 0.02^a	0.49 ± 0.02^a	0.49 ± 0.02^a
Weight gain (g)	1.97 ± 0.09^a	2.00 ± 0.22^a	2.06 ± 0.11^a	1.89 ± 0.26^a	1.97 ± 0.19^a
Growth rate (mg/d)	46.9 ± 3.78^a	47.6 ± 9.24^a	49.0 ± 4.62^a	45.0 ± 10.9^a	46.9 ± 7.98^a
SGR (%/day)	3.81 ± 0.11^a	3.88 ± 0.25^a	3.89 ± 0.05^a	3.77 ± 0.23^a	3.83 ± 0.17^a
Feed intake (g/shrimp)	4.06 ± 0.84^a	5.54 ± 0.98^a	5.52 ± 0.16^a	5.14 ± 0.76^a	5.22 ± 0.26^a
FCR	2.06 ± 0.33^a	2.77 ± 0.42^a	2.68 ± 0.32^a	2.72 ± 0.48^a	2.65 ± 0.43^a
Survival (%)	100 ± 0.0^a	99.6 ± 0.4^a	99.6 ± 0.4^a	99.1 ± 0.8^a	99.1 ± 0.8^a
PER	1.24 ± 0.19^a	0.92 ± 0.21^a	0.89 ± 0.14^a	0.90 ± 0.14^a	0.94 ± 0.17^a
APU (%)	56.7 ± 9.0^a	43.3 ± 8.0^a	44.7 ± 7.0^a	49.0 ± 8.0^a	52.0 ± 7.2^a

[1] SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio; APU, apparent protein utilization.

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Table 3. Response of juvenile *P. monodon* fed the soybean meal based diet (D0) and azolla meal based diet (D100) as a percentage of the total number of animals feeding on a diet during each replicate.

Replicate	Compartment X (D0) % Total	Compartment Y (D100) % Total
1	31	61
2	44	46
3	31	58
4	46	34
5	74	18
6	69	19
7	56	40
8	57	40
Mean ± SD	51 ± 16	40 ± 16

Discussion

The present study indicates that azolla meal prepared from aquatic plant of *Azolla pinnata* can replace up to 100% of soybean meal as a plant protein source in juvenile *P. monodon* diets without adverse effects on growth, survival, feed intake, FCR, PER, and APU. The present study suggested that azolla meal protein could be readily accepted by juvenile *P. monodon*, and hence feeding rate did not account for the growth reduction. Moreover, the results also suggest that total replacement of protein of soybean meal with azolla meal may provide optimal growth performance in cultured shrimp and it may be advantageous and lead to a reduction of feed costs. Of all plant proteins so far, soybean meal is the most promising source because of its high protein content and relatively balanced amino acid profile (Reigh and Ellis, 1992; Quartararo *et al*., 1998; Kikuchi, 1999). However, methionine and lysine are the most limiting amino acids in diets containing high levels of soybean meal (Andrews and Page, 1974; Lovell, 1989). Regardless the inferior nutritive value of soybean meal in terms of low methionine and lysine contents, in fact, the performance of soybean meal based diet (D0, 0% replacement) in the study was similar to that of azolla meal based diet (D100, 100% replacement). This will impact on enhancing the use of azolla meal as a major alternative plant protein source to soybean meal in diets for crustaceans.

Weight gain is affected by the quantity and quality of protein in the diet. Since the diets were calculated to be isonitrogenous and isolipidic (**Table 1**), the similar performance of shrimp fed the diet containing 100% soybean meal (D0, no azolla meal) and 100% replacement (D100, no soybean meal) may have resulted from superior quality of azolla meal. The high essential amino acid contents of methionine and lysine are believed to be superior nutritive value for penaeid shrimp diets as stated by Akiyama and Dominy (1991) and Millamena *et al.* (1996). However, inferior nutritive value for penaeid shrimp of formulated diets that have lower methionine (1.0-2.0% of dietary protein) and lysine (6.3-6.9% of dietary protein) levels than those of meat of clam and shrimp based diet (2.6-2.8% methionine; 7.3-8.9% lysine) was reported by Deshimaru and Shigeno (1972) and Deshimaru *et al*. (1985). A similar protein quality in terms of methionine and lysine contens in the diets containing different replacement levels of soybean meal protein by azolla meal may have resulted in no significant differences in growth performances of shrimp in the present study.

A similar growth rate (45-49 mg/d) observed in this study was achieved by shrimp fed all experimental diets regardless the replacement levels of dietary protein of soybean meal with azolla meal (**Table 2**). This growth rate is comparable to that observed by other previously workers using juvenile *P. monodon* under laboratory conditions. For example, Alava and Lim (1983) noted a 23.8 mg/d growth by 1.36 g shrimp fed a semipurified diet (40% protein) for 42 d, Pascual *et al.* (1983) reported a 11.7 mg/d increase in 1.76 g shrimp fed a semipurified diet (37% protein) for 42 d, while Bautista and Subosa (1997) reported a growth rate of approximately 70 mg/d by 4.8 g shrimp fed a shrimp-squid-shrimp head meal-based practical control diet (41% protein) for 60 d.

Feed intake is affected by palatability. Adequate palatability of soybean meal based diet has been demonstrated in crustaceans, such as *Macrobrachium rosenbergii* (Tidewell et al., 1993), juvenile *P. monodon* (Deshimaru *et al.*, 1985; Akiyama *et al.*, 1989; Pascual and Catacutan, 1990; Pascual *et al.*, 1990; Das *et al.*, 1992; Coman *et al.*, 1996; Penaflorida and Virtanen, 1996). No information is currently available on the palatability of azolla meal for shrimp species. Palatability of a diet is generally related to the content of dietary attractants. Amino acids such as alanine and glycine are good attractants for the penaeid shrimp (Clark et al., 1993; Coman et al., 1996). The present study indicates that there were no differences in palatability among the diets which the dietary major plant protein source levels of soybean meal were gradually replaced with azolla meal. Sources and contents of the attractants of both soybean meal and azolla meal may be similar. Proven data of the feeding preference test of the present study with no differences in % attractiveness (51% for soybean meal based diet vs 40% for azolla meal based diet) may have supported this explanation (*Table 3*).

CONCLUSIONS

The results of the present work demonstrate that azolla meal as a plant protein source can effectively replace up to 100% of protein from defatted soybean meal used in diets of juvenile *P. monodon* without any adverse effects on growth, survival, feed utilization efficiency, and palatability (feeding preference). It therefore appears that azolla meal prepared from *Azolla pinnata* has a potential role as another economical plant protein alternative to soybean meal in supplemental diet formulations for commercial production of black tiger shrimp (*P. monodon*).

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